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Chang, Michael

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Short Conceptual Overview

Long telomeres: too much of a good thing

Michael Chang

European Research Institute for the Biology of Ageing, University Medical Centre Groningen, University of Groningen, A. Deusinglaan 1, NL-9713 AV Groningen, The Netherlands

e-mail: m.chang@umcg.nl

Abstract

Telomeres, the physical ends of linear eukaryotic chromosomes, protect chromosome ends from end fusions and degradation. Telomere length is tightly regulated to ensure that telomeres are neither too short nor too long. Short telomeres are preferentially elongated by the enzyme telomerase. In the absence of telomerase, telomeres progressively shorten with each round of cell division. Critically shortened telomeres lose their ability to protect chromosome ends, inducing cell cycle arrest and senescence. While the consequences and cellular response to short telomeres are frequently explored, long telomeres also pose problems and cells have evolved mechanisms to shorten over-elongated telomeres. These aspects of long telomeres are discussed in this short conceptual overview.

Keywords: telomerase; telomere length regulation; telomere rapid deletion; telomeres.

Introduction

Eukaryotic DNA is organized into linear chromosomes. Maintaining the genetic information encoded within the DNA is an essential biological process. The DNA in our cells is constantly being challenged, both by DNA-damaging agents and by normal DNA metabolism, and any damage to the DNA must be repaired to safeguard the integrity of the genome. Perhaps the most hazardous DNA lesion is a double-stranded DNA break (DSB). DSBs, created by mechanical stress or DNA-damaging agents, need to be recognized and accurately repaired (1). In contrast, natural chromosome ends must be shielded from repair activities. Failure to do so could lead to cell cycle arrest, end-to-end fusion events, and loss of genome integrity. To combat this problem, cells have evolved specialized proteins that bind to short, repetitive, G-rich sequences at chromosome ends, forming protective nucleoprotein complexes called telomeres (2). However, the canonical DNA replication machinery is unable to fully replicate chromosome ends, resulting in telomere erosion with each round of cell division (3). In the vast majority of eukaryotes, telomere shortening is counteracted by a specialized reverse transcriptase called telomerase, whose core consists of a protein catalytic subunit and an RNA moiety, hTERT and hTR, respectively, in humans (4–6). Telomerase extends a telomere by repeated reverse transcription of a short sequence to the 3′ end of the telomere, using the RNA subunit as a template (7–9). The DNA replication machinery that is responsible for lagging strand synthesis presumably fills in the complementary 5′ strand. To ensure that telomeres are never in danger of becoming too short, telomerase preferentially extends short telomeres, an evolutionarily conserved feature of telomerase that has been observed in the budding yeast *Saccharomyces cerevisiae* (10), mice (11), and human fibroblasts expressing telomerase (12). Individuals born with reduced telomerase activity have short telomeres, which leads to telomere dysfunction in highly proliferative cells, and several human diseases are associated with shortened telomeres (13). Furthermore, critically shortened, dysfunctional telomeres are unstable and lead to chromosome end-to-end fusion events and genome instability, which can promote tumor progression (14, 15).

A review on telomeres typically includes a discussion on the consequences of harboring short telomeres, and a description of how cells recognize and extend short telomeres. However, telomere length is tightly regulated, not just to ensure that telomeres do not become too short, but also to prevent them from becoming over-elongated. If short telomeres can have such negative consequences, why are longer telomeres not evolutionarily selected? Can longer-than-normal telomeres have detrimental effects as well? This overview aims to highlight some of the important aspects of long telomeres.

Evolutionary considerations of long telomeres

Most human somatic cells do not express telomerase, resulting in progressive telomere shortening with each round of cell division (16). Extensively eroded telomeres trigger a DNA damage checkpoint response, which arrests cell cycle progression and causes cells to either die by apoptosis or enter a state known as replicative senescence (9, 17, 18). A recent report indicates that the presence of approximately five dysfunctional telomeres causes p53-dependent senescence in human cells (19). Senescence limits replicative potential and therefore has been proposed to be a cause of human aging, but it is also thought that replicative senescence is an important

barrier to tumorigenesis as cancer cells need to maintain their telomeres to continue proliferating. Thus, inheriting long telomeres may increase replicative potential, and perhaps life span, but it could result in increased cancer rates (20). However, this model seems unlikely as it has recently been noted that longer blood and epithelial cell telomere length is rarely associated with increased rates of cancer (21). On the other hand, short dysfunctional telomeres promote genome instability, which is a hallmark of cancer cells, and short telomeres are often linked to increased cancer risks (14, 15, 21). Furthermore, most cancers occur late in life when the force of selection pressure is reduced (21). Therefore, it is unlikely that individuals with long telomeres are selected against because of increased cancer rates.

An alternative explanation for keeping telomere lengths in check is the 'thrifty telomere' hypothesis, which suggests that long telomeres require more energy to maintain (21). In this model, telomeres should be kept as short as possible provided they are still fully functional. However, the evolutionary reasons for limiting telomere length are still far from being understood. Evolutionary models must be able to account for telomere length variation within a species as well as variation between species, and such models are often difficult to prove. Thus, instead of examining the strengths and weaknesses of these speculative models, this review will focus on the cellular and molecular consequences of long telomeres.

DNA replication stress at telomeres

Telomeric DNA sequences are highly repetitive and GC rich – features that are typically troublesome for the DNA replication machinery during DNA synthesis. Moreover, human telomeres are hypersensitive to UV-induced DNA damage (22), and sites of damage also cause problems for DNA polymerases. In *S. cerevisiae*, replication forks pause while traversing the telomeric repeats, and the strength of this pausing is proportional to telomere length (23, 24). Several proteins have been identified that promote telomeric DNA replication, suggesting that there may be multiple reasons for replication fork pausing at telomeres. The Rrm3 helicase promotes replication through the telomere, likely by facilitating replication past non-histone protein-DNA complexes (23, 25). In the fission yeast *Schizosaccharomyces pombe*, the telomere-binding protein Taz1 promotes DNA replication at telomeres (26). Similarly, mammalian telomeres also pose a challenge to the DNA replication machinery and require the Taz1-homolog TRF1 for efficient replication (27).

The telomeric DNA from most eukaryotic organisms can form G-quadruplex (G4) structures *in vitro* (28, 29). G4 DNA was first observed *in vivo* through studies using anti-G4 DNA-specific antibodies to detect such structures at ciliate telomeres (30, 31). In theory, the formation of G4 DNA structures should be problematic for a passing replication fork. In *S. cerevisiae*, the Pif1 helicase is needed to resolve G4 DNA, and in cells lacking Pif1, DNA replication is impeded and there is an increase in replication fork collapse (32). Other helicases, such as mammalian BLM, WRN, and RTEL, have also been implicated in promoting telomeric replication through the removal of G4 DNA structures (27, 33, 34). Taz1 and TRF1 have both been suggested to promote telomeric replication by recruiting one or more of these helicases (26, 27).

Increasing the length of a telomere would obviously increase the number of potential barriers to efficient telomeric DNA synthesis, making long telomeres more difficult to fully replicate. Furthermore, although there is some evidence that DNA replication can initiate within the telomeric tracts (27, 35), the majority of telomere replication is accomplished by replication forks originating from subtelomeric regions (24, 27). Thus, increasing the length of a telomere would also increase the distance that the replication fork must travel to fully replicate the telomere, which may result in a prolonged S phase and disruption of cell cycle progression. Taken together, cells may not favor the presence of long telomeres due to problems associated with the telomere replication.

Effect of long telomeres on telomere-binding proteins

Telomeric repeats are bound by telomere-binding proteins, so long telomeres would recruit more of these proteins. If cells maintain longer-than-normal telomeres, sufficient quantities of these telomere-binding proteins must be present to ensure proper capping of the telomeres. Remarkably, both *S. cerevisiae* and human cells can be manipulated to have extremely elongated telomeres with no dramatic effect on cell viability, indicating that telomere-binding proteins are not easily titrated below a threshold needed to maintain essential telomere capping (36, 37).

However, many telomere-binding proteins have both telomeric and non-telomeric functions. For example, *S. cerevisiae* Rap1 binds telomeric repeat DNA and is important to establish telomere length homeostasis (38, 39), but it was first discovered as a protein than can modulate gene expression (40). Furthermore, the Rap1-associated proteins Sir2, Sir3, and Sir4 are important for repressing transcription near telomeres as well as at the silent mating type loci (41). Sir2 is also important for regulating replicative life span through its role at the ribosomal DNA (rDNA) repeats (42, 43). Increasing telomere length may sequester telomere-binding proteins at the telomere and away from non-telomeric sites. Indeed, it has been reported that long telomeres reduce life span by reducing the amount of Sir2 available at the rDNA repeats (44). Similarly, long telomeres increase telomeric silencing, but reduce silencing at the *HMR* mating type locus by sequestering the Sir proteins to telomeres (45, 46). Whether the sequestration of telomere-binding proteins at over-elongated telomeres significantly affects other aspects of cell biology is still largely unknown.

Shortening over-elongated telomeres

The previous sections described potential downsides of harboring long telomeres. So how do cells shorten over-

elongated telomeres? The most obvious mechanism is to limit telomerase-mediated telomere extension (Figure 1A). Short telomeres are preferentially elongated by telomerase in *S. cerevisiae* (10), mice (11), and human fibroblasts expressing telomerase (12). In other words, long telomeres are less likely to be extended by telomerase and will progressively shorten at a rate similar to when telomerase is absent. Indeed, in *S. cerevisiae*, an artificially over-elongated telomere shortens at a rate of ~3–4 base pairs (bp) per generation, and this rate is independent of the presence or absence of telomerase (47). The shortening rate is ~50–150 bp per generation in mouse cells lacking telomerase (48), and in a variety of human cell types with no detectable telomerase activity (18, 49–51).

The reason for this shortening is primarily due to a combination of incomplete DNA replication of chromosome ends and nucleolytic degradation. Incomplete DNA replication occurs because (i) DNA polymerases can only synthesize DNA in a 5′ to 3′ direction, and (ii) DNA polymerases cannot synthesize DNA *de novo* and require a short primer of ~10 nucleotides (nt) of RNA. On the leading strand, DNA polymerase can theoretically synthesize DNA until it reaches the end of the chromosome, producing a blunt end. However, lagging strand synthesis occurs discontinuously, with each fragment (or Okazaki fragment) beginning with a short RNA primer. The RNA primers must be removed and replaced with DNA, which is synthesized by DNA polymerase from an upstream Okazaki fragment. Removal of the RNA primer of the terminal Okazaki fragment leaves a gap of ~10 nt that cannot be replaced. Without a mechanism to compensate for this loss, chromosome ends will progressively shorten. This phenomenon was termed the 'end-replication problem' (52, 53). In addition, to the end-replication problem, chromosome ends terminate with 3′ overhangs, the size of which varies from species to species. Thus, nucleolytic degradation must occur, at least on the blunt-ended telomere synthesized by the leading strand (3). The combined effect of the end-replication problem and nucleolytic degradation is that telomeres shorten with each round of cell division.

DNA damage can also contribute to the shortening of telomeres (Figure 1B). For example, the accumulation of single-stranded breaks in telomeric DNA, induced by oxidative stress, is a major cause of telomere shortening in human fibroblasts (54, 55). Furthermore, as mentioned above, DNA replication forks have difficulty traversing telomeric DNA (23, 24, 26, 27). If a replication fork collapses before reaching the end of the telomere, there is no replication origin distal to the site of fork collapse to generate a fork to finish the replication of the telomere, resulting in a truncated telomere (Figure 1C). Evidence for such truncated telomeres has been observed in *S. cerevisiae* (56). Normally, if the truncated telomere is significantly shortened, the telomerase will preferentially elongate it (10). However, if the telomere was initially over-elongated, the truncation may still leave the telomere longer than wild-type length. Telomerase would not preferentially elongate such a telomere, and this might be used as a mechanism to shorten over-elongated telomeres.

While incomplete replication, nucleolytic degradation, DNA damage, and replication fork collapse can all shorten over-elongated telomeres, these mechanisms act at telomeres of all lengths. In contrast, over-elongated telomeres are also

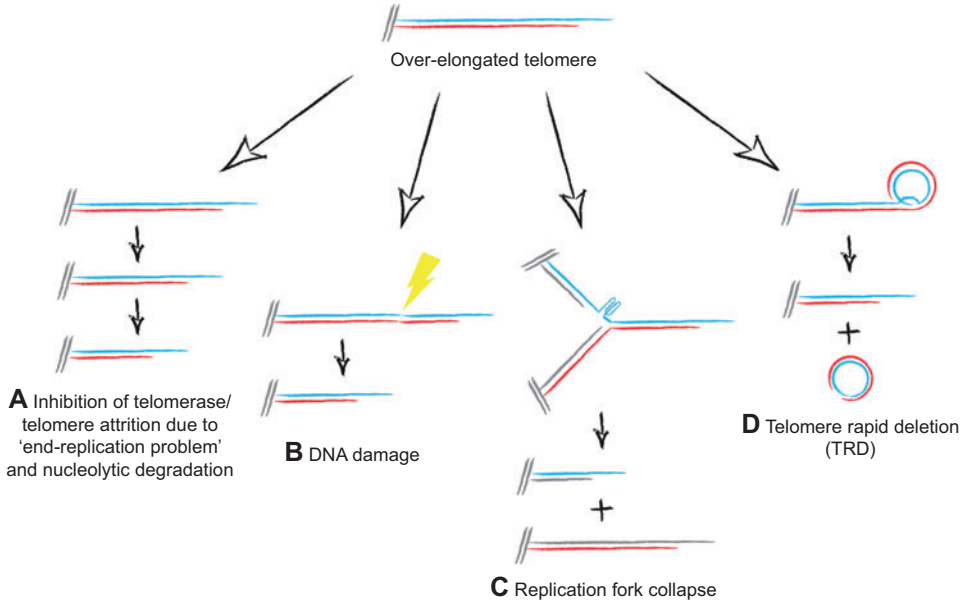

Figure 1 Shortening over-elongated telomeres.
(A) In the absence of telomerase, or when telomerase activity is inhibited, telomeres shorten due to a combination of incomplete DNA replication and nucleolytic degradation. (B) DNA damage within the telomeric tracts can lead to telomere truncation events. (C) Replication forks pause while traversing telomeric repeats. The fork pausing can be induced by proteins bound to the telomere or, as depicted here, by the presence of G4 DNA. Collapse of a stalled replication fork can lead to a truncated telomere. (D) Over-elongated telomeres can undergo TRD events, which return the telomere to approximately wild-type length by excising an extrachromosomal DNA circle containing telomeric repeats.

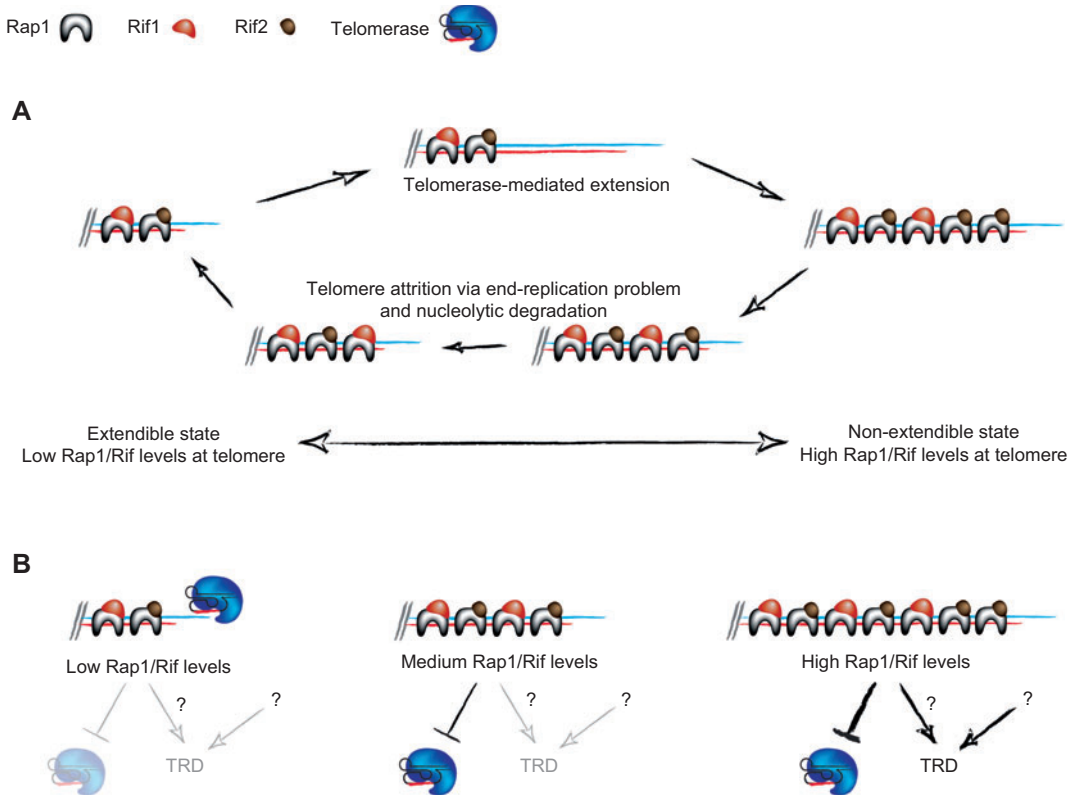

Figure 2 Regulating telomere length via a protein-counting mechanism in *S. cerevisiae*.
(A) Telomerase activity is regulated by telomere length, which is determined by the amount of Rap1, Rif1, and Rif2 bound at a telomere. A short telomere recruits few Rap1/Rif complexes, which favors telomerase-mediated extension of the telomere. A long telomere associates with many Rap1/Rif complexes, which inhibits telomerase activity. (B) While telomere-bound Rap1/Rif complexes regulate telomerase activity, it is unclear whether Rap1/Rif complexes can also mark a telomere for shortening by TRD. It is possible that TRD is regulated by a Rap1/Rif-independent mechanism.

specifically targeted for shortening by a mechanism called 'telomere rapid deletion' (TRD; Figure 1D) (57), which has also been referred to as 'telomere trimming' to avoid implying that the telomeres are completely deleted (58). TRD was first identified in *S. cerevisiae*, where it was shown that over-elongated telomeres could be shortened to approximately wild-type telomere length via a single intrachromosomal recombination event between telomere repeats (59, 60). A TRD event involves the excision of a telomere loop formed by the invasion of the telomeric 3′ overhang into telomeric sequence further upstream in the telomere. TRD has also been observed in *Kluyveromyces lactis* (61), *Arabidopsis thaliana* (62), and human cells (58, 63), indicating that it is a general mechanism for rapidly shortening over-elongated telomeres.

Telomere length determination

Having discussed how cells shorten over-elongated telomeres, an important question still remains: how do cells determine if a telomere is over-elongated? Telomere length determination is best understood in *S. cerevisiae*. Rap1 binds to double-stranded telomeric repeats about once every 18 bp (64). Rap1 recruits two additional proteins, Rif1 and Rif2, which act synergistically to negatively regulate telomerase (65, 66). Thus, the longer the telomere, the more Rap1/Rif1 and Rap1/Rif2 complexes will be at the telomere and the stronger the inhibition on telomerase (Figure 2A). Tethering Rap1, Rif1, or Rif2 to a telomere shortens the telomere in a manner that is proportional to the number of tethered molecules (38, 39). In human cells, a similar 'protein-counting' mechanism was also observed by targeting the telomeric proteins TRF1 and TRF2 to specific telomeres (67).

Although the precise details still need to be worked out, much is already known about the mechanisms by which short telomeres activate telomerase. Both physical and genetic evidence indicates that the Rif proteins inhibit Tel1, the yeast ortholog of human ataxia telangiectasia mutated (ATM), which is a positive regulator of telomerase (68, 69). When a telomere is short, there are fewer Rap1/Rif complexes at the telomere, allowing Tel1 to act. Indeed, recruitment of Tel1 to telomeres is about 10-fold higher at short telomeres than at wild-type length telomeres (70). Tel1 is a kinase and its kinase activity is important for its role in telomere length maintenance (71), but there is currently no consensus on what its critical telomeric phosphorylation targets are.

Considerably less is known about the targeting of over-elongated telomeres for shortening. As mentioned above, it

is known that telomerase activity is inhibited at long telomeres, but it is unclear whether they are specifically targeted for shortening by TRD, and if they are, whether a similar Rap1/Rif protein-counting mechanism is employed (Figure 2B). It is also unclear whether the frequency of TRD is directly proportional to telomere length. However, TRD is a recombination-mediated event, and recombination efficiency is directly proportional to the length of the substrate DNA in prokaryotes, yeast, and mammalian cells (72–77). Consistent with this notion, it has been observed, under certain circumstances, that long telomeres in *S. cerevisiae* preferentially undergo recombination (78). It will be interesting to determine whether TRD is subject to active regulation, and if so, how this is accomplished.

Outlook

Although the consequences of long telomeres have less obvious impact than those of short telomeres, it is clear that over-elongated telomeres must be shortened. More work is needed to characterize the cellular response to over-elongated telomeres, and to determine whether long telomeres are subject to evolutionary selection pressure. Such work is particularly important given the connections between telomere length homeostasis and human health.

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Conflict of interest statement

The author declares that no conflict of interest exists.

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Michael Chang received his PhD degree at the University of Toronto in 2005 under the supervision of Dr. Grant W. Brown, studying DNA damage response pathways using high-throughput functional genomics. After his PhD, he took a position at the Swiss Institute for Experimental Cancer Research in Lausanne as a postdoctoral fellow in the lab of Dr. Joachim Lingner, whose research is focused on telomerase and chromosome end replication. In 2008, Michael moved to the lab of Dr. Rodney Rothstein at the Columbia University Medical Center in New York, where he continued to study factors that regulate telomerase as well as telomerase-independent mechanisms of telomere maintenance. In 2011, he joined the European Research Institute for the Biology of Ageing in the Netherlands as an Assistant Professor. Work in his lab focuses on telomere maintenance and genome integrity as it relates to cancer and aging.